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3. Janicke et al, Sem. Throm. Hemostasis, 1991, 17:303-312)

4. Nekarda et al (Cancer Res., 1994, 54:2900-2907)

5. Grandahl-Hansen et al, 1993, Cancer Research 53:1513-1521)

6. JOURNAL OF NEURO-ONCOLOGY, (1994) Vol. 22, No. 2, pp. 139-151.

7. INTERNATIONAL JOURNAL OF ONCOLOGY, (MAR 1994) Vol. 4, No. 3, pp. 717-721.

8. Biol.Chem.Hoppe Seyler (376, No. 5, 259-67, 1995) 2 Fig. 67 Ref.

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Review

Plasminogen Activator Inhibitor Type 1 in Cancer: Therapeutic and Prognostic Implications

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Degradation of the extracellular matrix plays a crucial role in cancer invasion. This degradation is accomplished by the concerted action of several enzyme systems, including generation of the serine protease plasmin by the urokinase pathway of plasminogen activation, different types of collagenases and other metalloproteinases, and other extracellular enzymes. The degradative enzymes are involved also in tissue remodelling under non-malignant conditions, and the main difference appears to be that mechanisms which regulates these processes under normal conditions are defective in cancer.

Specific inhibitors have been identified for most of the proteolytic enzymes, e.g. plasminogen activator inhibitors (PAI's) and tissue inhibitors of metalloproteinases (TIMP's). It has been contemplated that these inhibitors counteracted the proteolytic activity of the enzymes, thereby inhibiting extracellular tissue degradation which in turn should prevent tumor cell invasion.

This review focuses on plasminogen inhibitor type 1 (PAI-1). It is described that PAI-1 is not produced by the epithelial cancer cell but by the stromal cells in the tumors, suggesting a concerted action between stroma and tumor cells in the processes controlling proteolysis in cancer. The specific localization of PAI-1 to the tumor stroma and in many cases to areas surrounding the tumor vessels has lead us to suggest that PAI-1 serves to protect the tumor stroma from the ongoing uPA-mediated proteolysis. This hypothesis is supported by recent clinical data showing increased levels of PAI-1 in metastases as compared to the primary tumor as well as data demonstrating that high levels of PAI-1 in tumor extracts from breast, lung, gastric and ovarian cancer is associated with a shorter overall survival. We further hypothesize that PAI-1 itself or uPA:PAI-1 interaction might represent an attractive new target for anti-invasive and antimetastatic therapy.

Key words: Cancer / Histology / PAI-1 / Prognosis / Therapy.

Introduction

Cancer invasion is a complex process in which degradation of the extracellular matrix plays a crucial role. This degradation is accomplished by the concerted action of several enzyme systems, including generation of the serine protease plasmin by the urokinase pathway of plasminogen activation (Danø *et al.*, 1984), different types of metalloproteinase, and other extracellular enzymes (Liotta *et al.*, 1991). These degradative enzyme systems are also involved in tissue remodelling under non-malignant conditions, and the main difference appears to be that the normal regulation of these processes is defective in cancer (Danø *et al.*, 1984; Blasi *et al.*, 1987; Liotta *et al.*, 1991; Pöllänen *et al.*, 1991).

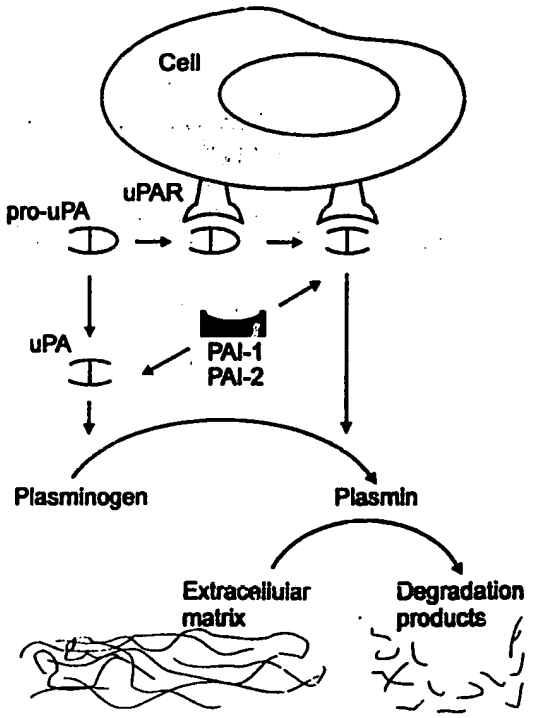

Fig. 1 Schematic Drawing of the uPA-Catalysed Plasminogen Activation and Degradation of Extracellular Matrix.

Our laboratory has mainly focused on the urokinase pathway of plasminogen activation, which is a quite complex cascade reaction (Figure 1). The urokinase-type plasminogen activator is synthesized as a virtually inactive proenzyme (pro-uPA), that requires proteolytic activation before it in turn can activate plasminogen. Pro-uPA activation is efficiently catalyzed by plasmin, leading to a strong amplification of the overall reaction (Danø *et al.*, 1984). It is not known how the cascade is initiated. A specific receptor for uPA (uPAR) localizes the enzyme on cell surfaces; concomitant binding of pro-uPA to this receptor and of plasminogen to as yet unidentified binding sites strongly enhances and focalizes plasmin generation (Danø *et al.*, 1994). Thus, the surface of uPAR expressing cells has been suggested to be the major site of plasmin formation.

Active uPA is inhibited by plasminogen activator inhibitors (PAIs). In order of reaction constants these are PAI-1, PAI-2, protease-nexin and PAI-3 (for review see Kruithof, 1988). PAI-1 is the principal physiological inhibitor of both tPA and uPA (Kruithof, 1988).

PAI-1

The human PAI-1 gene is located on the long arm of chromosome 7 in position q21.3-q22 (Ginsburg *et al.*, 1986; Klinger *et al.*, 1987). It covers 12 kb and consists of nine exons (Loskutoff *et al.*, 1987; Bosma *et al.*, 1988; Strandberg *et al.*, 1988). In humans two PAI-1 mRNAs, of approximately 2.4 and 3.4 kb, respectively, have been demonstrated (Andreasen *et al.*, 1986; Ginsburg *et al.*, 1986; Ny *et al.*, 1986; Pannekoek *et al.*, 1986). The two transcripts differ exclusively in the length of the 3' untranslated sequence being generated by alternate polyadenylation and splicing (Loskutoff *et al.*, 1987; Bosma *et al.*, 1988; Strandberg *et al.*, 1988). PAI-1 is a glycoprotein with a predicted glycosylated molecular mass of 52 kDa. The 379 amino acid sequence of mature PAI-1 includes three potential asparagine-linked glycosylation sites. There are no cysteines in the molecule (Ginsburg *et al.*, 1986; Ny *et al.*, 1986; Pannekoek *et al.*, 1986). Based on amino acid homology PAI-1 belongs to the serpin (serpin protease inhibitor) family of proteins (Potempa *et al.*, 1994).

Serpins inhibit their target proteases by providing a so-called 'bait' sequence that mimics the normal substrate of the protease. During the reaction between the inhibitor and its target serin protease, an equimolar, SDS-stable, inactive complex is generated. The reactive centre of PAI-1, located on a exposed loop, contains the 'bait' peptide bond between residues Arg^{346} and Met^{347} (Andreasen *et al.*, 1986). This bond mimics the bond of plasminogen which is cleaved by uPA during the conversion of plasminogen to the active enzyme plasmin.

PAI-1 is synthesized in an active form, however, it rapidly converts from the active state to an inactive latent form (Hekman and Loskutoff, 1988). The term latent originates from the fact that latent PAI-1 can be partially reactivated by exposure to various denaturants (Hekman and Loskutoff, 1985) or negatively charged phospholipids (Lambers *et al.*, 1987). The crystal structure of latent PAI-1 has been solved and shows that in the latent state the loop containing the reactive site bond is inaccessible to the protease because it is buried within the surface of PAI-1 (Mottonen *et al.*, 1992). PAI-1 also exists in a so-called substrate form. In contrast to active PAI-1, this form does not form stable inactive complexes with its target serin protease. Instead it is cleaved at the reactive centre peptide bond thereby releasing the carboxyterminal 33 amino acids of PAI-1 (Declerck *et al.*, 1992). Thus, due to conformational transition of PAI-1 four functionally different forms of PAI-1 exist.

PAI-1 occurs throughout the body with the highest concentrations observed in the liver and spleen. However, its concentration and, notably, its activity differs considerably from organ to organ (Simpson *et al.*, 1991). The expression of PAI-1 is regulated by a variety of cytokines, growth factors, hormones, phorbolesters and endotoxins (for review, see Andreasen *et al.*, 1990; Krishnamurti and Alving, 1992).

PAI-1 is synthesized *in vitro* by a variety of cell types including endothelial cells (Dawson and Henney, 1992). It is a major component of the extracellular matrix (ECM) and its activity is stabilized in the ECM by binding to the multifunctional protein vitronectin (Mimuro *et al.*, 1987; Mimuro and Loskutoff, 1989). Binding of PAI-1 to vitronectin does not interfere with the ability of vitronectin to promote adhesion and spreading of cells (Salonen *et al.*, 1989). Thus, it has been suggested that the interaction between PAI-1 and vitronectin may serve to confine pericellular uPA activity to focal contact sites where cells use proteolysis in regional detachment (Salonen *et al.*, 1989). Furthermore, PAI-1 inhibits receptor-bound uPA nearly as efficiently as uPA in solution (Ellis *et al.*, 1990). Thus, accumulation of active PAI-1 within the tissue may enable PAI-1 to play a major role in controlling cell-surface plasminogen activation and extracellular proteolytic activity, besides of being a key player in the control of the fibrinolytic system.

uPA/PAI-1 complexes bound to uPAR on cell surfaces binds to the multiligand receptors α_2-macroglobulin receptor/low density lipoprotein receptor-related protein (α_2-MR) and epithelial glycoprotein 330 (gp330). These receptors mediate endocytosis of uPA/PAI-1 complexes from the extracellular space and their degradation (Andreasen *et al.*, 1994).

In the ECM thrombin neutralizes PAI-1 that is complexed with vitronectin and may thus promote plasminogen activator activity (Ehrlich *et al.*, 1991). Activated protein C (APC) also neutralizes PAI-1 activity through the formation of APC/PAI-1 complexes (Krishnamurti and Alving, 1992). Both APC and thrombin, but also elastase, inactivate PAI-1 by limited proteolysis (de Fouw *et al.*, 1987; Levin and Santell, 1987).

Based on the spatial relationship between uPA- and PAI-1-expressing cells in the course of physiological angiogenesis, it seems likely that PAI-1 functions during angiogenesis to protect neovascularized tissues from ex-

cessive proteolysis (Bacharach *et al.*, 1992). However, the role of modulations of extracellular proteolytic activity in angiogenesis is likely to be varied and complex (Pepper *et al.*, 1994).

Localization of PAI-1 in Cancer

Lewis Lung Carcinoma

The first report in the literature on the immunolocalization of PAI-1 in cancer tissue described the presence of the protein in cancer cells in the murine Lewis lung carcinoma (Kristensen *et al.*, 1990). In this tumor, PAI-1 immunoreactivity was found in all primary tumors in a consistent pattern: in most areas, PAI-1 positivity was prominent in cancer cells and the staining was colocalized with uPA. In contrast, in the periphery of the tumors, where the tissue showed signs of ongoing destruction, PAI-1 staining was weak or absent, whereas uPA staining showed high intensity. These data, showing the ability of cancer cells to produce at the same time uPA enzyme and PAI-1, together with later histological studies of the same tumors showing the presence also of uPAR in cancer cells (Eriksen, personal communication), indicate that in Lewis lung carcinomas the malignant cells themselves are regulating the uPA-mediated proteolysis. However, as will be reviewed below, later studies of a number of different human tumors have revealed a more complex picture of how plasminogen activation is regulated in cancer tissue.

Colon Cancer

In colon adenocarcinomas, PAI-1 mRNA was detected by *in situ* hybridization in 14 samples of resected cancer (Pyke *et al.*, 1991a). Expression of PAI-1 was consistently seen in endothelial cells of small vessels in tumorous areas, whereas surrounding unaffected tissue was negative or only weakly positive. In addition, clusters of PAI-1 mRNA containing stromal cells distinct from endothelial cells were frequently observed in richly vascularized areas. The exact identity of these cells could not be established, but it was proposed that they might represent sprouting endothelial cells. The controls included the use of two different non-overlapping antisense probes that gave exactly the same result while both of the corresponding sense probes were negative in all cases. In agreement with this study, an immunohistochemical study of cryostat sections of colon cancer samples reported PAI-1 protein to be located in blood vessels in 7 out of 12 cases (Buø *et al.*, 1993). In the same study, however, also cancer cells were reported to be positive for PAI-1. It is possible that PAI-1 produced by the stromal cells form complexes with uPA and that these complexes then are internalized by the cancer cells, which are known to produce uPAR (Pyke *et al.*, 1991b). Another explanation for the different observations on PAI-1 localization in colon adenocarcinomas is that the reported localization in the cancer cells was not specific for PAI-1, since only one monoclonal anti-PAI-1 antibody was used in the study. Clearly, with respect to PAI-1 expression, colon adenocarcinomas are very different from Lewis lung carcinomas in that the stromal compartment contributes as the source of PAI-1 in the former.

It has been proposed that the function of PAI-1 in colon cancer is to protect the tumor stroma from degradation and in particular the newly formed vasculature of the tumor stroma (Pyke *et al.*, 1991a; Danø *et al.*, 1994).

Breast Cancer

In a histological study of ductal mammary carcinomas, we investigated samples by both *in situ* hybridization and immunohistochemistry for the presence of PAI-1 (Pyke *et al.*, manuscript in preparation). The two methodologies produced essentially the same results. Most prominent was the localization of PAI-1 mRNA and protein in stromal fibroblast-like cells surrounding malignant epithelium, while less than 5% of the cases contained PAI-1 mRNA or protein positivity in cancer cells. In most cases, endothelial cells of small vessels were clearly positive for PAI-1 (Figure 2). The main part of the immunostaining was reticular and associated with the extracellular matrix surrounding cancer cells. Positive control experiments included the use of two non-overlapping anti-sense RNA probes for *in situ* hybridization and 5 different monoclonal antibodies against PAI-1 for immunohistochemistry. This study is in some disagreement with another study of 43 human breast carcinomas using monoclonal antibodies against PAI-1 (Reilly *et al.*, 1992). First, Reilly *et al.* did not find stromal staining in any of the samples except for PAI-1 positive blood vessels. Secondly, in their study, cancer cells consistently were positive for PAI-1 as were epithelial cells in normal looking ducts. We never observed PAI-1 in the normal breast epithelium. There is no information given on the subtype of breast carcinomas studied by Reilly *et al.* (1992), but it is possible that different subtypes

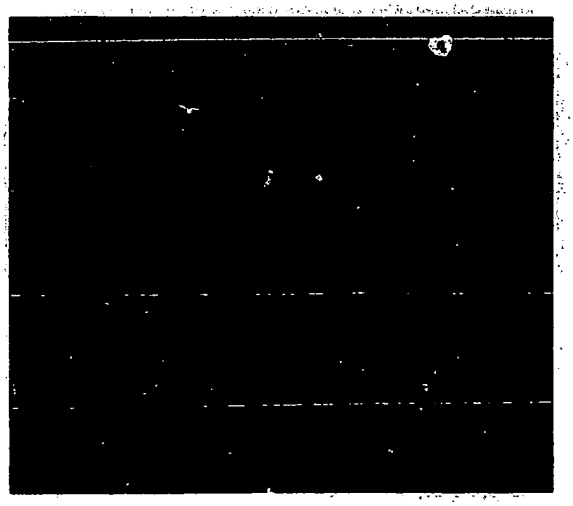

Fig. 2 Immunohistochemistry for PAI-1 in a Specimen of Ductal Mammary Carcinoma Showing Stromal Positivity in a Typical Basementmembrane-Like Pattern Surrounding the Malignant Cells.

may show considerable differences in PAI-1 expression patterns as has been observed for skin cancers of basal and squamous cell type (see below) and that this could account for the differences reported. Alternatively, differences in tissue processing during the immunohistochemical staining procedures could render different epitopes of the PAI-1 protein variably sensitive to immunodetection. It is known, for instance, that paraffin embedded tissue is inferior to frozen tissue as regards immunodetection of a great variety of antigens. Thus it is possible that the absence of staining in stromal elements reported in an immunohistochemical study of paraffin sections from benign breast disease and breast cancers is a consequence of a decreased staining sensitivity (Jankun *et al.*, 1993).

Skin Cancer

Sappino *et al.* (1991) reported the presence of PAI-1 mRNA in cancer cells in 10 out of 10 cases of squamous cell carcinoma of the skin. Similar data have been obtained by us (Pyke, unpublished data). Thus, these cancers resemble Lewis lung carcinomas with respect to the expression of PAI-1 in cancer cells. In contrast, no signal for PAI-1 mRNA was found in basal cell carcinomas of the skin (Sappino *et al.*, 1991). In melanocytic neoplasia, two recent reports describe the localization of PAI-1 mRNA (Delbaldo *et al.*, 1994; de Vries *et al.*, 1994) in melanoma cells in advanced melanomas but no signal in benign and early stages of melanocytic neoplasia. The majority of the PAI-1 protein was reported to be located extracellularly around tumor cells (de Vries *et al.*, 1994) similarly to the pattern of PAI-1 immunostaining reported for mammary tumors (Pyke *et al.*, manuscript in preparation).

Brain Tumors

Three recently published studies report on the localization of PAI-1 in brain tumors. Kono *et al.* (1994) used a polyclonal antibody to rat PAI-1 crossreactive with human PAI-1 on sections of human brain tumor samples. They consistently found PAI-1 protein in proliferative vessels of high-grade gliomas and metastatic tumors, while tumor cells were never found to contain PAI-1. Based on their findings and in agreement with the findings of PAI-1 localization in colon and mammary cancers referred above, the authors propose that PAI-1 in these cancers may be involved in angiogenesis. In another study *in situ* hybridization with digoxigenin-labelled oligonucleotide probes was used to determine the localization of PAI-1 mRNA in brain tumors (Yamamoto *et al.*, 1994). Positive signals were found in glioblastoma cells and were described to be particularly strong around sites of vascular proliferation. Apart from that, endothelial cells in the tumor tissue also contained PAI-1 mRNA. Normal brain tissue and low-grade tumors were negative or only weakly positive for PAI-1. In some contrast to these two reports the third study describes the immunolocalization of PAI-1 protein in cancer cells in glioblastomas but not in endothelial cells (Caccamo *et al.*, 1994).

Other Cancer Types

Using monoclonal anti-PAI-1 antibodies on paraffin embedded histological specimens, cancers of the lung (Gris *et al.*, 1993), liver (Fitch *et al.*, 1994) and pancreas (Takeuchi *et al.*, 1993) have been reported to express PAI-1 in cancer cells mainly, but as stated above caution should be taken when interpreting immunostaining data obtained from paraffin sections.

Despite some discrepancies between some of the histological data referred here, it is evident that in several human cancers only stromal cells produce PAI-1 (e.g. colon adenocarcinomas), while in others only cancer cells make PAI-1 (e.g. skin squamous cell carcinomas). This suggests that there is a tissue specific regulation of the PAI-1 gene in the cancerous tissue, as has also been reported for the uPA and uPAR genes (Pyke *et al.*, 1991b; Danø *et al.*, 1994).

Therapeutic Implications of Inhibiting PAI-1

In adenocarcinoma of the colon and breast and in glioblastomas PAI-1 is predominantly expressed by the stroma cells including the endothelial cells lining the newly formed tumor vessels, while no PAI-1 mRNA or protein is seen outside the tumor. High tumor PAI-1 content has been shown to predict a poor prognosis in patients with breast, lung, gastric and ovarian cancer, and metastases appear to have higher PAI-1 content than the corresponding primary tumors (Sier *et al.*, 1994).

The role of PAI-1 in tumor growth and invasion may thus be more complex than previously believed, and PAI-1 may in fact be a tumor growth promoting factor in certain cancer types. In this sense, PAI-1 could contribute to the processes that eventually lead to invasion and metastasis. A surplus of PAI-1 in the extracellular matrix inside the tumor could serve to protect the tumor stroma from autodegradation by urokinase-catalyzed plasmin formation. In the formation of metastasis PAI-1 might be of importance for reimplanting of circulating tumor cells, since formation of new stroma at the metastatic site would require the blockade of uPA mediated degradation of the extracellular matrix.

This has lead us to suggest that inhibition of PAI-1 may represent a new strategy in cancer therapy. Treatment with inhibitors of uPA:PAI-1 binding would disturb the local balance between plasminogen activation and inhibition and result in initiation of autodegradation of the tumor stroma and specifically the pericapillary matrix by excess urokinase found in the invasive areas, which is responsible for plasmin activation and thereby proteolysis. Degradation of the pericapillary stroma will eventually be detrimental for tumor angiogenesis. An antiangiogenic effect will not only reduce growth of the primary tumor, but may also inhibit the formation of metastasis.

The realization of the involvement of stromal cells in cancer progression represents a new paradigm in tumor biology which opens the prospect of new therapeutic ap-

proaches directed against this involvement, e.g. by interfering with the signals which the cancer cells use to recruit/induce the stromal cells and by the use of drugs that are either selectively toxic for the relevant stromal cells or selectively inhibit the transcription of the proteolytic components produced by these cells.

In analogy with the clinical findings we have shown that mouse PAI-1 mRNA is expressed in the stromal cells of metastasizing human xenografts in athymic nude mice (Rømer, personal communication). Thus, this model can be used to test the raised hypothesis on anti-PAI-1 based therapy. Also tumor studies in PAI-1 (–/–) knock out mice (Carmeliet *et al.*, 1993) may contribute to a better understanding of the role of PAI-1 in tumor progression.

Prognostic Implications of PAI-1

In the majority of human cancers including malignant melanoma, breast, colon, bladder, prostate and lung cancer, there is a critical need for the development of new independent prognostic markers. The challenge is to identify those patients at low risk from those at high risk of recurrence. An accurate means of distinguishing between these two risk groups would spare the majority of these patients from severe side effects of adjuvant chemotherapy and make more efficient use of health resources. Also useful would be the development of tumor markers for screening of total populations and high-risk groups, for diagnosis as an aid in staging or confirmation of histopathology, and in therapy for predicting drug response and monitoring relapse.

Since it is well established that proteolytic activity is necessary for tumor cell spreading, molecules involved in the regulation of invasion and metastasis are attractive as prognostic/diagnostic tools. Several investigators have reported increased tumor and plasma PAI-1 levels in cancer patients as compared to normal healthy controls (Fucre *et al.*, 1991; Sumiyoshi *et al.*, 1991; Sier *et al.*, 1994; Kuhn *et al.*, 1995; Casslén *et al.*, 1994), suggesting that the analysis of PAI-1 may yield valuable prognostic/diagnostic information.

In the published studies different buffers have been used for the preparation of the extracts on which the protein and PAI-1 analyses have been performed. The buffers can be grouped as follows: a neutral buffer with detergent (Jänicke *et al.*, 1993, 1994; Nekarda *et al.*, 1994; Ganesh *et al.*, 1994), a neutral buffer without detergent (steroid receptor buffer) (Grøndahl-Hansen *et al.*, 1993; Foekens *et al.*, 1994; Jänicke *et al.*, 1994), and an acid buffer with detergent (Pedersen *et al.*, 1994a, b). The type of extraction buffer to be used is probably less important for PAI-1 than for uPA. Jänicke *et al.* (1994) found that addition of the detergent Triton X-100 to the extraction buffer resulted in a two-fold increase in the uPA concentration (ng/mg protein) while the PAI-1 concentration remained almost the same. The protein concentration was about 12% higher in the Triton X-100 extracts. Similarly we found that the uPA concentration in extracts made with an acid buffer containing detergent compared with a neutral buffer without detergent was about 8-fold higher, while the PAI-1 concentration remained unchanged (Rosenquist *et al.*, 1993 and unpublished). Based on these results it is tempting to conclude that it is of no importance which buffer is used for extraction of PAI-1. However, since PAI-1 forms complexes with uPA, it cannot be excluded that some PAI-1 assays could be sensitive to the varying amounts of uPA present. Furthermore, some assays could also be sensitive to other changes imposed on the PAI-1 molecule by the specific extraction buffers.

In the studies published on the prognostic impact of tumor PAI-1, four different sandwich PAI-1 ELISAs and at least three different PAI-1 standards have been used. The different ELISAs use different combinations of antibodies: monoclonal/monoclonal (Jänicke *et al.*, 1993; 1994; Grøndahl-Hansen *et al.*, 1993, Nekarda *et al.*, 1994, Pedersen *et al.*, 1994a, b), monoclonal/polyclonal (Ganesh *et al.*, 1994), polyclonal/monoclonal (Foekens *et al.*, 1994). Even though it is unlikely that these ELISAs measure exactly the same components in the extracts, they all have been prover valuable in predicting prognosis. They truly all measure PAI-1, but some of the forms of PAI-1 (latent/active, free or in complex with either uPA or tPA, or in complex with vitronectin) may be measured with different sensitivity in the different assays. Thus, since it is likely that the extraction procedure is influencing the composition of the different forms of PAI-1 in the extracts, each different ELISA might have its own preferable extraction procedure.

It is not possible at the moment to recommend a specific combination of PAI-1 assay, protein determination, PAI-1 and protein standard and extraction method. In order to harmonize studies on PAI-1 tumor content, an international standard for PAI-1 and quality ensured reference material have been established (BIOMED 1, manuscript in preparation). Future publications should contain information on the concentration of PAI-1 per mg protein in the reference tumor extract, measured against the international standard of PAI-1 and the international standard for protein, obtained with the PAI-1 method and the protein assay used by the laboratory in question. Conversion factors between the international standard and (if different) the standards used in the study for both protein and PAI-1 should be included. Obeying these simple rules will allow comparison between studies even though they are conducted with different assays.

Breast Cancer

Fucre *et al.* (1991) found a 74-fold increase of PAI-1 in breast tumors as compared to normal breast tissue. Similar findings were reported by Sumiyoshi *et al.*, 1991 who also found that increased levels of tumor PAI-1 were directly proportional to the number of tumor positive axillary lymph nodes.

Jänicke *et al.* (1991) were the first to describe the prognostic role of PAI-1 in breast tumor extracts. Including

tumor extracts from 113 breast cancer patients, high tumor PAI-1 content as determined by a sandwich ELISA was shown to be an independent and significant predictor of poor prognosis. Two later studies by Grøndahl-Hansen *et al.* (1993) including 190 patients and by Foekens *et al.* (1994) including 657 patients confirmed the prognostic impact of PAI-1 in patients with node negative and node positive breast cancer.

Of particular interest is that PAI-1 seems to be an independent prognostic variable, i.e. measurement of tumor PAI-1 content contributes significantly to the prognostic information which can be obtained by other prognostic parameters. For example, in the subgroup of patients with estrogen receptor positive tumors, who have a better prognosis than patients with estrogen receptor negative tumors, PAI-1 tumor measurements allows for a further prognostic stratification (Grøndahl-Hansen *et al.*, 1993). Also in the subgroup of patients with 1–3 tumor positive axillary lymph nodes, PAI-1 could be used to separate the patients into significantly different prognostic groups (Grøndahl-Hansen *et al.*, 1993). This latter observation suggests that in the group of patients with 1–3 tumor positive lymph nodes a subgroup of high-risk patients can be identified and these women might be offered more intensive chemotherapy.

In the study by Foekens *et al.* (1994) PAI-1 appeared to be the strongest biochemical prognostic marker when uPA, cathepsin D, pS2, estrogen and progesterone receptors were included as the other biochemical variables, indicating the importance of PAI-1 measurements in predicting prognosis in breast cancer.

Gastric Cancer

Analyzing PAI-1 tumor content in 76 patients with complete resection of their gastric cancer, Nekarda *et al.* (1994) were able to demonstrate prognostic significance of PAI-1, high PAI-1 being significantly associated with poor prognosis when using the best cut-off value to stratify the patients in two groups, 45 patients having low values and 31 having high values. In a multivariate Cox regression analysis, PAI-1 was proven to be an independent prognostic factor, with nodal status and WHO classification as the two only other prognostic factors.

Pancreatic Cancer

Applying immunohistochemistry on paraffine sections, Takeuchi *et al.* (1993) studied the prognostic role of tumor PAI-1 and PAI-2 staining intensity in 97 patients with pancreatic cancer. While strong staining intensity for PAI-2 was significantly associated with long overall survival, PAI-1 staining intensity had no impact on survival.

Colon Cancer

Tumor PAI-1 levels as measured by ELISA are found significantly elevated in primary colon adenocarcinomas and their metastasis as compared to normal colon mucosa: normal mucosa < primary tumor < liver metastasis (Sier *et al.*, 1994). The authors conclude that the high PAI-1 content in colorectal cancer metastasis in the liver is associated with an inactivation of the enhanced urokinase cascade, which might allow tumor cells to settle in the liver. Ganesh *et al.* (1994) studied the prognostic impact of PAI-1 in 92 colon carcinomas and found no significant correlation between PAI-1 as determined by sandwich ELISA and patient outcome. However, it should be mentioned that the biopsies used for this study were all obtained from the center of the tumors (Verspaget, personal communication), while we previously reported that PAI-1 as determined by *in situ* hybridization (Pyke *et al.*, 1991a) is predominantly expressed in the tumor stroma in the near vicinity of the invasive front, i.e. at the periphery of the tumor.

Lung Cancer

In a retrospective study including tumor tissue from 106 lung adenocarcinoma patients we determined PAI-1 by sandwich ELISA. Using the upper and lower quartiles as cut-off points, high PAI-1 was shown to be significantly ($P = 0.017$) correlated with short overall survival (Pedersen *et al.*, 1994a). In Cox multivariate analysis including clinical parameters and tumor uPA, PAI-1 was shown to be an independent prognostic marker for survival, stage being the only other significant prognostic factor. When analyzing the 69 stage I patients separately and using the median as cut-off point, high levels of PAI-1 were significantly ($P = 0.038$) associated with poor prognosis.

In a second retrospective study including tumor tissue from 84 patients with squamous cell lung cancer and 38 patients with large cell lung cancer (Pedersen *et al.*, 1994b), there was a non-significant trend towards high PAI-1 levels being associated with poor prognosis in squamous cell lung cancer. However, combining high tumor levels of PAI-1 and high tumor levels of urokinase-type plasminogen activator receptor (27 of the 84 patients), a highly significant ($P = 0.008$) association with short survival was seen. PAI-1 did not have any significant correlation to survival in the group of large cell lung cancer patients (Pedersen *et al.*, 1994b).

Ovarian Cancer

A number of studies have shown that tumor concentration of PAI-1 in ovarian cancers is significantly higher as compared with benign ovarian tissue specimens (Casslén *et al.*, 1994; Kuhn *et al.*, 1995).

In a recent study by Kuhn *et al.* (1995), PAI-1 as determined by ELISA was shown to predict survival in advanced ovarian cancer patients after radical surgery and platinum-based chemotherapy, i.e. high tumor levels of PAI-1 were significantly ($P = 0.01$) associated with short survival. A best cut-off point was defined dividing the patients into a group of 27 with low and 24 with high PAI-1 tumor levels. In the multivariate analysis residual tumor after operation and high PAI-1 or high uPA were the only prognostic factors.

Plasma PAI-1

PAI-1 can also be detected in plasma and has been shown to be elevated in patients with pancreatic cancer (Sandberg *et al.*, 1992), ovarian cancer (Casslén, 1994) and in urinary tract cancers (Bashar *et al.*, 1994). In the last study, plasma levels were significantly higher in a group of patients with metastatic disease than in patients without distant metastasis. An association between the degree of cancer cell atypia and plasma PAI-1 levels was reported.

Conclusion

PAI-1 is consistently expressed at invasive foci in many types of human cancer, while no PAI-1 mRNA or protein is seen outside the tumor tissue. In adenocarcinoma of the colon and breast and in glioblastomas PAI-1 is predominantly expressed by the stromal cells including the cells forming the tumor vessels. In squamous cell carcinoma of the skin PAI-1 is expressed by the tumor cells. We hypothesize that PAI-1 serves to protect the tumor cells and the tumor stroma from the extensive uPA-mediated tissue degradation the tumor imposes on itself. This stromal cell interaction represents a new paradigm with important implications for basic cancer biology and cancer treatment.

It should be emphasized that the exact identification of PAI-1 expressing cells in different cancer forms must continue to have a high priority for several reasons. First, the unravelling of which cell types produce which components of the plasminogen activation system may yield information on how proteolysis is regulated in cancers *in vivo*. Secondly, and this is especially true for cancers in which stromal cells are found to produce PAI-1, the positive correlation between high extractable levels of PAI-1 and poor prognosis seen in several cancers strongly suggests that some stromal cell types are assisting the cancer cells in the invasion and that these cells, once identified might represent targets for therapeutic intervention.

Treatment with inhibitors of PAI-1 or uPA:PAI-1 interaction would disturb the local balance between plasminogen activation and inhibition and result in autodegradation of the tumor stroma.

High levels of PAI-1 in breast, lung, gastric and ovarian cancer appear to be highly significant and independent predictors of short survival. PAI-1 tumor measurements may hence be used to identify patients who should be offered intensive chemotherapy, or patients who could be spared the toxic anti-neoplastic treatment. Since cancer patients may have elevated PAI-1 plasma levels, it should be studied whether measurements of plasma PAI-1 in cancer patients will provide the same prognostic information as when measuring tumor PAI-1.

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